

26. (Reiterated) The method of Claim 1, wherein culturing a proliferating epidermal basal cell population comprising one or more epidermal basal cell(s) comprises separating basal cells from keratinocytes using a calcium-free medium.

27. (Reiterated) The method of Claim 1, wherein said antisense oligonucleotide(s) is modified with one or more thio groups.

### REMARKS

#### The Pending Claims

Prior to entry of the above amendments, Claims 1-27 are pending. Claims 1-4 and 26-27 are directed to a method of transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell. Claims 5-10 are directed to a transdifferentiated cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell. Claims 11 and 12 are directed to a kit for converting epidermal basal cells into cells having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell. Claim 13 relates to a method of using transdifferentiated epidermal basal cells having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell to isolate a novel nerve growth factor. Claim 14 relates to a method of using transdifferentiated epidermal basal cells having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell to screen a potential new drug for treating a nervous system disorder. Claims 15-24 are directed to a transdifferentiated epidermal basal cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal

or glial cell. Claim 25 relates to a cell culture derived from the transdifferentiated cell of Claim 15.

The Office Action, Telephonic Examiner Interview on December 20, 2001, and Applicants' Amendment

In the Final Office Action mailed July 17, 2001, no claims were allowed. Additionally, acknowledgement was made therein of Applicants' Response to Notice of Non-Compliant Amendment, which Applicants mailed on April 27, 2001, which referred to the remarks filed in Applicants' prior Response to Office Action, which Applicant mailed on March 21, 2001.

On December 20, 2001, Examiner M. Schmidt and Supervisory Patent Examiner J. LeGuyader, graciously granted Applicant's undersigned attorney a telephonic interview with the participation of one of the co-inventors Dr. Toomas Neuman. Applicants again thank the Examiners for their kindness and attention in granting the interview. In the interview, Applicants' undersigned attorney stated Applicants' desired to address the Examiner's grounds of rejection perhaps more clearly than Applicants were able to do in their prior Response to Office Action.

A. Double Patenting Rejections

Claims 1-27 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-15 of U. S. Patent No. 6,087,168 for the same reasons of record as set forth in the Official Action mailed 11/21/00.

In the Final Office Action, and again in the telephonic interview, on December 20, 2001, the Examiner acknowledged Applicants' stated willingness to file a terminal disclaimer in compliance with 37 CFR 1.321 (c) to overcome an actual or provisional rejection based on a nonstatutory double patenting ground, which Applicants stated in their Response to Office Action, mailed on March 21, 2001. Applicants reassert here that upon receipt of written

confirmation of allowable subject matter, Applicants will file a terminal disclaimer, since the conflicting application or patent is commonly owned with the above-captioned application.

B. Rejections Under 35 USC § 112

(i) The Examiner rejected Claims 5-12 and 15-25 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described adequately in the specification for the same reasons of record as set forth in the Official Action mailed 11/21/00 as well as the following reasons:

In response [to Applicant's prior arguments], the written description rejection was made based on the variability known in the art for neuronal cell morphology and physiology. It was argued that one skilled in the art would not be in possession of the scope of possible "neuronal" cells claimed in view of the breadth of claimed "neuronal" cells. It was argued that the specification as filed, although it teaches expression of certain markers, does not provide a representative number of species of possible "neuronal" cells broadly encompassed by any cell expressing any neural-specific antigenic marker known in the art. Since the claimed cells are generated recombinantly, via addition of certain growth factors and antisense to reduce gene expression of certain genes, the claimed "transdifferentiated" cells would not be expected to share the same gene expression patterns as nerve cells generated naturally in a whole organism. In fact, the claimed cells initiate as differentiated epidermal cells, which would have a specific pattern of epidermal cell gene expression prior to manipulation with the nerve growth factors and antisense as taught in the specification as filed. Thus, to assert that any known nerve-antigenic marker would serve as a marker for the claimed "transdifferentiated" cells would not take into account the unique nature of the present invention, wherein the antisense to human MSX1 and human HES1 genes, coupled with growth in particular nerve growth factors, results in a specific phenotype of cells as taught in the specification as filed. One skilled in the art would have no grounds for the assumption that any neuronal marker would be generated under these conditions as applied to epidermal cells, considering the unpredictability in the art as to expression patterns in different nerve cells at different stages of development and the inability to draw a specific nexus between the expression patterns in nerve cells developing naturally in a whole organism with the "neuronal" cells of the claimed invention which begin as differentiated epidermal cells.

In response, and as discussed in the Examiner Interview on December 20, 2001, to address the Examiners' concerns regarding the scope of claimed cells, Applicants have amended Claim 5 to include the limitations of Claim 6 related to a range of markers that are expressed by the claimed transdifferentiated cell, i.e., "... at least one marker selected from the group consisting of nestin, neural RNA-binding protein Musashi, neurofilament M, neural-specific  $\beta$ -

tubulin, neural-specific enolase, microtubule associated protein 2, glial fibrillary acidic protein (GFAP), O4, or a combination of any of these". Also, for greater clarity, the word "neurotrophin" is now replaced by the phrase "signal molecule selected from the group consisting of BDNF, CNTF, NGF, NT-3, NT-4, IL-6, sonic hedgehog, and sonic hedgehog aminoterminal peptide". Support for these amendments to Claim 5 can be found, e.g., in Claims 1 and 6, as originally filed, and in the specification.

For instance, growth in the presence of a signal molecule is discussed in the specification. Support for the recitation of the phrase "signal molecule" itself is found in the specification, e.g., at page 18, lines 13-15. Signal molecules, such as BDNF, CNTF, NGF, NT-3, NT-4, IL-6, and sonic hedgehog, are disclosed in Claim 1, as originally filed, as well as in the specification at page 15 lines 13-25 and page 17 lines 15-20. Support for the recitation of "sonic hedgehog aminoterminal peptide" is found in the specification, e.g., at page 15, lines 18-21. The recited expression markers are disclosed in Claim 6, as originally filed, as well as in the specification, e.g., at page 18 lines 8-9 and 18-22.

Moreover, the specification contains extensive and detailed guidance to a wide array of useful markers and other methods for detecting features characteristic of neural progenitors, neuronal and glial cells. For instance, the specifications disclose numerous useful examples of morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell and methods for detecting them such as the discussion of detection methods in the specification at page 15 line 30 through page 17 line 2 and page 17 line 21 through page 18 line 22. While working examples are not required, the specification also discloses working examples of how various neural-specific antigenic markers were detected on the surfaces of transfected cells (specification, page 27 line 22 to page 28 line 2).

Applicants have cancelled Claims 6 and 15, which are made redundant by the amendment of Claim 5, with respect to the recited markers. Claims 16, 17, 21, 22, 23, and 25 have been amended to depend from Claim 8 instead of canceled Claim 15.

Applicants believe these amendments overcome this ground of rejection, and Examiner Schmidt stated to Applicants' undersigned attorney during the telephonic Examiner Interview that in view of such amendments, she would look toward an allowance.

(ii) The Examiner also rejected Claims 1-27 under 35 U.S.C. 112, first paragraph, for the following reasons:

In response [to Applicant's prior arguments], the claims are drawn to transdifferentiated cells and methods of using said cells. The claims are not drawn to methods for screening for cells which produce at least one neuronal antigenic marker. Thus, to make and use the claimed invention, one skilled in the art must be able to first make the claimed cells. Applicant argues that the method used to generate the transdifferentiated cells encompasses a mixed group of cells in cell culture isolated from human skin, and that the actual characterization of the cells is not important for the use of the cells to screen potential drug targets as long as the cells have at least one feature of a neuronal cell. For the reasons argued above, Examiner disagrees that Applicant would be in possession of a representative number of such transgenic cells by merely recognizing one possible antigenic marker known in the art for any possible type of neuronal cell. The specification as filed teaches that certain antigenic markers are expressed and certain phenotypes are encompassed. The claim of the specific phenotypic markers serves to identify the cells characterized by the specification as filed as "neuronal" since the art does not teach an art defined term for such dedifferentiated cells. The need to perform further basic research to characterize other such transdifferentiated cells would involve further experimentation. Since neither the art nor the specification as filed teach specific guidance as to what other possible neuronal antigenic markers would characterize the claimed cells as "neuronal", one skilled in the art would necessarily practice "trial and error" experimentation to further characterize any possible cell as transdifferentiated as broadly claimed. In view of the lack of guidance in the art, one skilled in the art would necessarily practice an undue amount of experimentation to make and use the claimed invention.

In response, and as discussed in the telephonic Examiner Interview, on December 20, 2001, Applicants have amended Claim 1 to insert a step (e), which includes the limitations of Claim 3 related to a range of markers that are expressed by the transdifferentiated cell in accordance with the claimed method. Also, in step (d) of Claim 1, the word "neurotrophin" is now replaced by the word "signal molecule", and in the Markush group, the phrase "sonic hedgehog aminoterminal peptide" is recited, while the phrase "active fragments of any of these" is deleted. In addition, the Markush language in step (b) of Claim 1 is clarified by the insertion of the word "selected" before "from the group".

Support for the recitation of the word “signal molecule” itself is found in the specification, e.g., at page 18, lines 13-15. Growth in the presence of a signal molecule is discussed in the specification; signal molecules, such as BDNF, CNTF, NGF, NT-3, NT-4, IL-6, and sonic hedgehog, are disclosed in Claim 1 as well as in the specification at page 15 lines 13-25 and page 17 lines 15-20. Support for the recitation of “sonic hedgehog aminoterminal peptide” is found in the specification, e.g., at page 15, lines 18-21. The recited expression markers, i.e., “. . . at least one marker selected from the group consisting of nestin, neural RNA-binding protein Musashi, neurofilament M, neural-specific  $\beta$ -tubulin, neural-specific enolase, microtubule associated protein 2, glial fibrillary acidic protein (GFAP), O4, or a combination of any of these”, are disclosed in Claim 3, as originally filed, as well as in the specification, e.g., at page 18 lines 8-9 and 18-22.

Also regarding Claims 1-27, the Examiner noted that claim amendments in Applicant's Response to the prior Office Action clarified the negative regulators claimed but the Examiner asserted that the claims as written “do not specify *in vitro* and thus still broadly read on *in vivo* use.” Examiner Schmidt and Supervisory Patent Examiner LaGuyader indicated their approval of Applicants' proposed amendment of Claims 1, 11, and 25, to amend the claim preambles to clarify the *in vitro* nature of the claimed method.

Applicants believe the amendment of Claim 1, combined with the amendments to Claims 5, 11, 16, 17, 21, 22, 23 and 25, discussed above, remove the bases of the Examiner's rejection and make Claims 1, 2, 4, 5, 7-12, 17-27 ready for allowance. Examiner Schmidt stated to Applicants' undersigned attorney during the telephonic Examiner Interview, on December 20, 2001, that in view of such amendments, she would look toward an allowance.

Applicant has canceled Claims 13 and 14, without prejudice. Therefore, the rejection of Claims 13 and 14 is mooted. The cancellation of Claims 13 and 14 is made with the reservation of Applicants' right to pursue the subject matter of these claims in a continuation application, which right was acknowledged by the Examiner during the interview.

**CONCLUSION**

In view of the above amendments and remarks and as indicated by the Examiner during the interview by the Examiner, it is submitted that this application is now ready for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,

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**Version with Markings to Show Changes Made**

**In the Claims:**

Please cancel Claims 3, 6, 13, 14 and 15, without prejudice. Please amend Claims 1, 5, 11, 16, 17, 21, 22, 23 and 25 as follows.

1. (Twice Amended) An in vitro method of transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell, comprising:

(a) culturing a proliferating epidermal basal cell population comprising one or more epidermal basal cell(s), said cell(s) derived from the skin of a mammalian subject;

(b) transfecting said epidermal basal cell, in vitro, with one or more eukaryotic expression vector(s) containing at least one cDNA encoding a human neurogenic transcription factor, or homologous non-human counterpart, or active fragment(s) thereof, selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, such that at least one of the neurogenic transcription factor(s) is expressed in said cell;

(c) growing the transfected cell in the presence of at least one antisense oligonucleotide comprising a segment of a human MSX1 gene and/or human HES1 gene, or homologous non-human counterpart of either of these, in an amount sufficient to suppress the expression of functional MSX1 gene product and/or HES1 gene product; [and, optionally,]

(d) growing said epidermal cell with a retinoid and at least one [neurotrophin]signal molecule selected from the group consisting of BDNF, CNTF, PDGF, NGF, NT-3, NT-4, sonic hedgehog, and sonic hedgehog aminoterminal peptide[active fragments of any of these], or a cytokine comprising IL-6, whereby the cell is transdifferentiated into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell; and

(e) wherein the physiological and/or immunological feature is expression of a marker



selected from the group consisting of nestin, neural RNA-binding protein Musashi, neurofilament M, neural-specific  $\beta$ -tubulin, neural-specific enolase, microtubule associated protein 2, glial fibrillary acidic protein (GFAP), O4, or a combination of any of these.

2. (Reiterated) The method of Claim 1, wherein the eukaryotic expression vector(s) of the transfection step comprise a CMV promoter sequence operatively linked to a DNA(s) encoding the neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, and wherein the DNA encoding the neurogenic transcription factor is of human origin or is a homologous non-human counterpart, or is an active fragment of a gene encoding any of these.

3. Canceled.

4. (Reiterated) The method of Claim 1, wherein the morphological feature comprises one or more morphological neurite-like process(es) at least about 50 micrometers in length.

5. (Amended) A transdifferentiated cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell, comprising:

an epidermal basal cell transfected with one or more expression vectors comprising a CMV promoter sequence operatively linked to a DNA(s) encoding the neurogenic transcription factor NeuroD1, NeuroD2, ASH1, Zic1, Zic3, or MyT1, wherein the DNA encoding the neurogenic transcription factor is of human origin, or is a non-human homologous counterpart, or is an active fragment of a gene encoding any of these, said cell being treated with at least one antisense oligonucleotide comprising a segment(s) of human MSX1 gene or human HES1 gene, or non-human homologous counterpart thereof, and wherein said cell was grown in the presence of a retinoid and at least one [neurotrophin] signal molecule selected from the group consisting of BDNF, CNTF, NGF, NT-3, NT-4, IL-6, sonic hedgehog, and sonic hedgehog aminoterminal

peptide, thereby transdifferentiating said epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell said cell expressing at least one marker selected from the group consisting of nestin, neural RNA-binding protein Musashi, neurofilament M, neural-specific  $\beta$ -tubulin, neural-specific enolase, microtubule associated protein 2, glial fibrillary acidic protein (GFAP), O4, or a combination of any of these.

6. Canceled.

7. (Reiterated) The transdifferentiated cell of Claim 5, wherein the morphological feature comprises one or more morphological neurite-like process(es) at least about 50 micrometers in length.

8. (Reiterated) A transdifferentiated cell produced by the process of Claim 1.

9. (Reiterated) The transdifferentiated cell of Claim 8, wherein the physiological and/or immunological feature expressed by the cell is a marker selected from the group consisting of nestin, neural RNA-binding protein Musashi, neurofilament M, neural-specific  $\beta$ -tubulin, neural-specific enolase, microtubule associated protein 2, glial fibrillary acidic protein (GFAP), O4, or a combination of any of these.

10. (Reiterated) The transdifferentiated cell of Claim 8, wherein the morphological feature expressed by the cell is one or more morphological neurite-like process(es) at least about 50 micrometers in length.

11. (Twice Amended) A kit for converting in vitro epidermal basal cells into cells having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell, said kit comprising:

(a) one or more eukaryotic expression vector(s) containing cDNA encoding a neurogenic transcription factor, or fragment thereof, from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, or a non-human homologous counterpart of any of these;

(b) at least one antisense oligonucleotide corresponding to the human MSX1 gene, the human HES1 gene, or a non-human homologous counterpart of either of these; and

(c) a retinoid and at least one [neurotrophin] signal molecule selected from the group consisting of BDNF, CNTF, PDGF, NGF, NT-3, NT-4, [and] sonic hedgehog, and sonic hedgehog aminoterminal peptide.

12. (Reiterated) The kit of Claim 11, further comprising instructions for using (A), (B), and (C) in transdifferentiating a mammalian subject's epidermal basal cell(s).

13. Canceled.

14. Canceled.

15. Canceled.

16. (Amended) The transdifferentiated cell of Claim [15] §, wherein the cell further displays the physiological feature of a lack of mitotic activity under cell culture conditions which induce differentiation in neural progenitor cells.

17. (Amended) The cell of Claim [15] §, wherein the transdifferentiated cell has a morphological, physiological, or immunological feature specific to a neuronal cell.

18. (Reiterated) The transdifferentiated cell of Claim 17, wherein the physiological and/or immunological feature is expression of neural RNA-binding protein Musashi, neurofilament M, neural-specific  $\beta$ -tubulin, neural-specific enolase, microtubule associated

protein 2.

19. (Reiterated) The transdifferentiated cell of Claim 17, wherein the cell is a GABAergic cell.

20. (Reiterated) The transdifferentiated cell of Claim 17, wherein the cell is a dopaminergic cell.

21. (Amended) The transdifferentiated cell of Claim [15] 8, wherein the morphological feature is one or more neurite-like process(es) at least about 50 micrometers in length.

22. (Amended) The transdifferentiated cell of Claim [15] 8, wherein the cell is of human origin.

23. (Amended) The cell of Claim [15] 8, wherein the transdifferentiated cell has a morphological, physiological, or immunological feature specific to an astroglial or oligodendroglial cell.

24. (Reiterated) The transdifferentiated cell of Claim 23, wherein the physiological and/or immunological feature is expression of glial fibrillary acidic protein (GFAP) or O4.

25. (Amended) An in vitro cell culture derived from the transdifferentiated cell of Claim [15] 8, comprising a plurality of cells that express one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell.

26. (Reiterated) The method of Claim 1, wherein culturing a proliferating epidermal basal cell population comprising one or more epidermal basal cell(s) comprises separating basal cells from keratinocytes using a calcium-free medium.

27. (Reiterated) The method of Claim 1, wherein said antisense oligonucleotide(s) is modified with one or more thio groups.